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Selective Complexation of *N*-Alkylpyridinium Salts: Recognition of NAD^+ in Water

Christian Jasper, Thomas Schrader,* Jens Panitzky, and Frank-Gerrit Klärner*

N-Alkylpyridinium salts are widely used in nature in coenzyme-mediated redox processes; the oxidized form of nicotinamide adenine dinucleotide (NAD^+) formally abstracts a hydride ion from other biomolecules and is converted into its reduced form, NADH. Efficient and selective molecular recognition of this important class of compounds by artificial receptor molecules opens up the possibilities of influencing biological redox processes, alteration of the NAD^+ redox potential,[1] targeted transport of NAD^+, attachment of NAD^+ to artificial enzymes, and many others. In water, the challenge in constructing such a synthetic receptor molecule was to find a way to replace the highly stable solvation shell around this organic cation with attractive, intermolecular, noncovalent receptor – substrate interactions. Salt bridges alone are not sufficient, because of the energetic costs of anion and cation desolvation. Much more appropriate is the cation – π interaction, because only the cation has to be dehydrated prior to complexation and, in water, this interaction is also supported by the hydrophobic effect.[2] This major noncovalent force has recently been discovered in numerous natural recognition events[3] and has been intensively studied by several groups.[4] These groups found that macrocycles containing aromatic units are able to completely desolvate a number of biologically important organic cations, for example, quaternary ammonium ions,[5] guanidinium ions,[6] and *N*-alkylpyridinium ions.[7] Usually, however, these macrocyclic receptors are not very selective towards their substrate topology. To date, for example, such receptors cannot distinguish between ball-shaped or planar structures.

Molecular clips with planar side walls should be much more shape-selective toward flat structures. Porphyrin clips derived from diphenylglycoluril are capable of complexing *N*-alkylpyridinium cations (for example, viologen cations),[8] but they also bind to electron-rich aromatic compounds such as pyridine, hydroxypyridine, and phenols.[9] To date, such clips have been only studied in organic solvents.[8] Recently, a new molecular clip has been introduced that features two face-to-face naphthalene systems, held apart at a maximum distance of 10 Å by a rigid substituted benzene spacer (Figure 1).[10, 11]

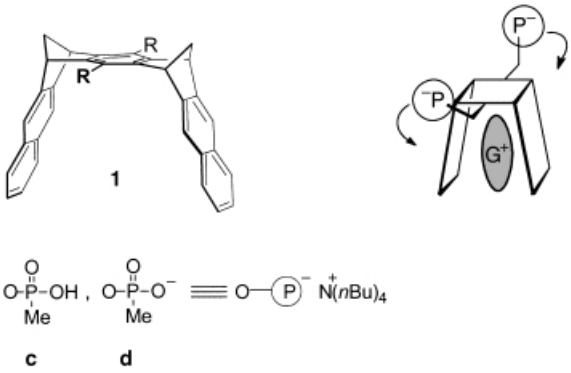

Figure 1. Left: Structures of the molecular clips **1a** – **d**. Right: Design of the new hybrid receptor system by combination of an electron-rich molecular clip and a bisphosphonate tweezer.

Single-crystal structural analyses of various complexes revealed that the aromatic walls of the clip embrace the substrate molecule tightly, by reducing the distance between the naphthalene tips from about 10 to 8 Å,[10] as shown in the single-crystal structure of the 1:1 complex between *N*-ethyl-4-carbethoxypyridinium triiodide and the diacetoxy-substituted clip **1a** (Figure 2). Herein, we report the synthesis of the water-soluble molecular clip **1d** and its intriguing receptor properties in aqueous solution.

The synthesis of the clip molecule **1d** began with the reaction of the hydroquinone **1b** with two equivalents of

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Supporting information for this article is available on the WWW under http://www.angewandte.com or from the author.
NAD = nicotinamide adenine dinucleotide

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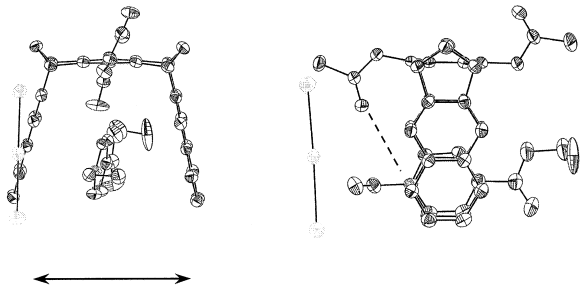


Δd = –1.7 Å (from 10.0 to 8.3 Å)

Figure 2. Single-crystal structure of the 1:1 complex between *N*-ethyl-4-carbethoxypyridinium triiodide and **1a**.[10]

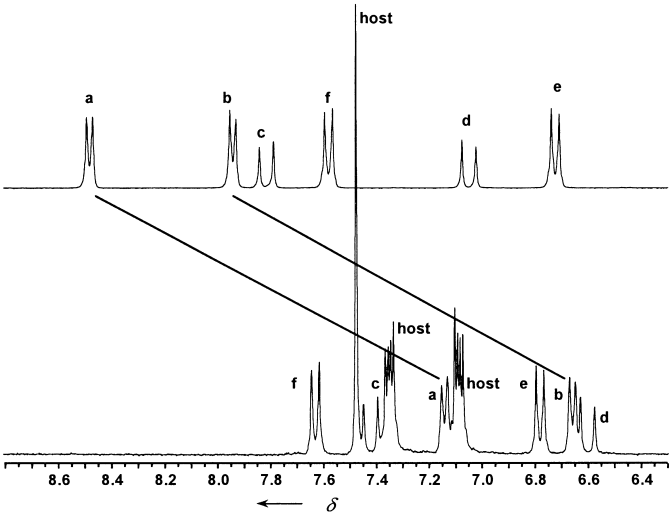
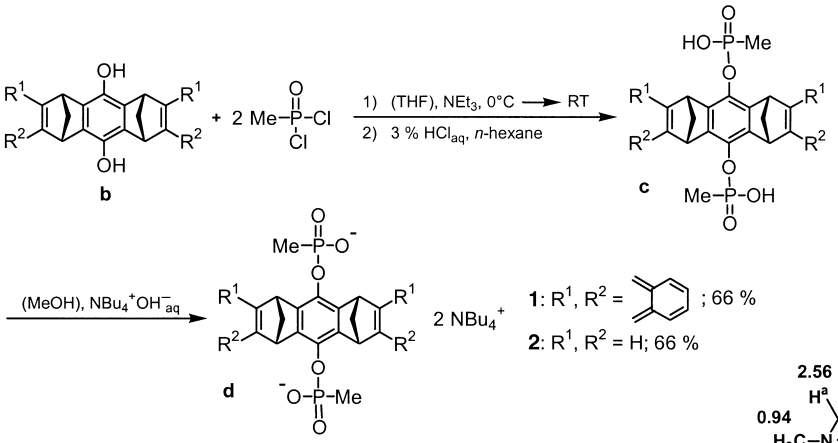


Figure 3. ^{1}H NMR spectra (aromatic region) of **3** before and after addition of **1d** in methanol. Note the strong upfield shifts of the pyridinium protons a and b (see Scheme 2 for the assignment of the substrate protons).

methylphosphonic acid dichloride, followed by hydrolytic work-up (Scheme 1). The free phosphonic acid **1c** was purified by flash column chromatography and subsequently converted into its tetrabutylammonium salt **1d**. From hydroquinone **2b**, an analogous preparation led to **2d**, which is

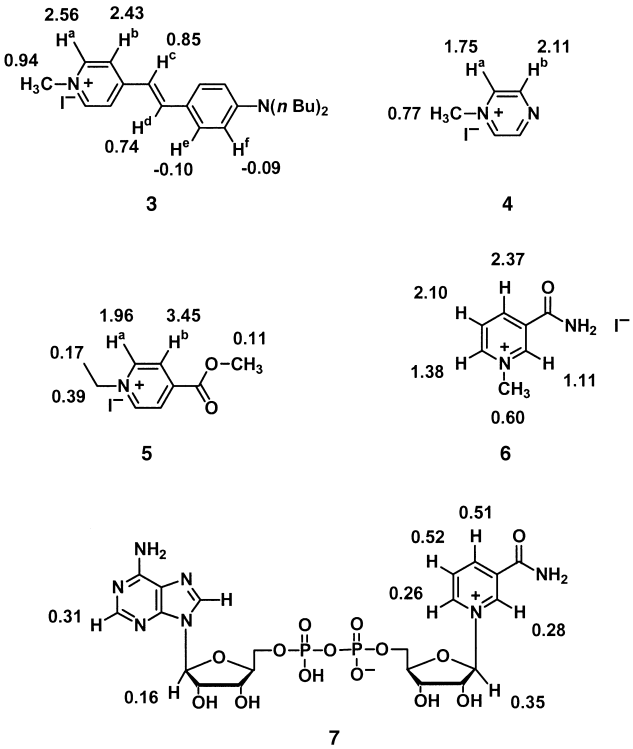


Scheme 1. Synthesis of the molecular clip **1d** and the model compound **2d**.

structurally related to **1d** but lacks the naphthalene walls. This simple model compound (**2d**) served as a reference and an aid in the elucidation of the binding mode of **1d**. Both **1d** and **2d** are very soluble in a wide range of solvents of differing polarities, ranging from chloroform to water.

Clip **1d** combines two powerful noncovalent binding motifs; the clip cavity and the two phosphonate moieties, attached to the hydroquinone spacer unit, which act like the tips of a tweezer. This arrangement mimics the *p*-xylylene bisphosphonates, whose efficiency in ammonium and guanidinium binding is already well established.[12]

The magnetic anisotropy of the three arene units of **1** makes ^{1}H NMR spectroscopy a very sensitive probe for detecting the complexation of a substrate molecule inside the cavity of **1**. Stoichiometric (1:1) mixtures of **1d** and the *N*-alkylpyridinium salts **3**–**7** in methanolic or aqueous solution displayed pronounced complexation-induced upfield shifts of the signals from the substrate protons (Figure 3, Scheme 2), whereas the effect of complexation on the ^{1}H NMR shifts of the signals arising from the receptor protons is smaller but still significant. This effect is of comparable size in all complexation studies discussed here, and was therefore employed for the quantitative analysis of the titrations.[13] The maximum complexation-induced upfield shifts $\Delta\delta_{max}$, the association constants K_a, and hence the Gibbs enthalpies of complexation ΔG, were determined at 20 °C by ^{1}H NMR dilution titrations, in which the dependencies of the complexation-induced shifts $\Delta\delta$ of the signals from the protons of receptor **1d** on different substrate concentrations were meas-



Scheme 2. Substrate structures and the maximum complexation-induced ^{1}H NMR upfield shifts ($\Delta\delta_{max}$) of 1:1 complexes of guests **3**–**7** with **1d** in water (**3**: methanol).

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ured. For the complexation of *N*-methylnicotinamide (**6**) with **1d**, the complex stoichiometry was determined to be 1:1, by use of a Job plot. In each 1:1 mixture of the model compound **2d** with **3**–**7**, the chemical shifts of the protons of **3**–**7**, as well as those of **2d**, remained completely unchanged, which indicates that in these cases no complexes were formed.

From the data listed in Table 1, it becomes evident that **1d** forms surprisingly stable complexes with **3**–**7** in the protic solvents methanol and water compared, for example, with the complex formed between **1a** and the Kosower salt **5** in $CDCl_3$, for which the association constant and the complexation-induced shifts in ^{1}H NMR resonance signals were determined by titration to be $K_a = 137 \pm 14\,\text{M}^{-1}$ ($\Delta G = -2.9 \pm 0.1$ kcal mol^{-1}), and $\Delta\delta_{max} = 1.82$ (**5**;H^a) and 2.40 (**5**;H^b) at 21 °C, which indicates a moderate stability. The observation that the complexes formed in water ($K_a = 4800 - 12\,700\,\text{M}^{-1}$)

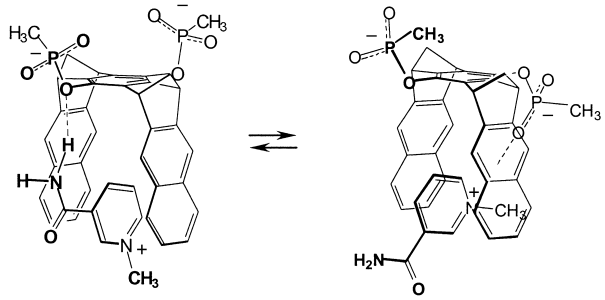
Table 1. Association constant K_a and Gibbs enthalpy of association ΔG for complex formation between **1d** and **3**–**7**, determined by ^{1}H NMR dilution titrations in methanol and water at 20 °C.

Substrate	K_a [M^{-1}][a,b] in CD_3OD	$-\Delta G$ [kcal mol^{-1}]	K_a [M^{-1}][a,b] in D_2O	$-\Delta G$ [kcal mol^{-1}]
3	4000 ± 990	4.8	broad signals[c]	–
4	600 ± 30	3.8	9400 ± 2260	5.3
5	3200 ± 350	4.7	4800 ± 720	4.9
6	6900 ± 540	5.2	12700 ± 3840	5.5
7	precipitation[c]	–	6500 ± 620	5.1

[a] Determined from the complexation-induced shifts of the same three (in CD_3OD: the same four) receptor signals. In some cases, the analysis of the dependence of $\Delta\delta$ of substrate protons on the concentration of **1d** produced larger K_a values than the concentration dependence of the signals of **1d**; the given values represent the lowest limits for all binding constants. [b] The given errors are standard deviations between the K_a values from different NMR signals; the standard deviations from the nonlinear regressions were consistently lower. [c] Precipation of the complex after mixing **1d** and **7**, or broad signals in the ^{1}H NMR spectrum of a mixture of **1d** and **3**, prevented the determination of K_a.

are even more stable than the corresponding ones formed in methanol ($K_a = 600 - 6900\,\text{M}^{-1}$) is good evidence for the substantial contribution of hydrophobic interactions in the receptor–substrate binding processes observed here. This observation implies that, especially in water, it is the cation–π interactions and not the salt bridges that are largely responsible for complex stability. A distinct downfield shift of the resonance signal arising from the methyl group of the phosphonate in **1d** (0.2–0.4 ppm) on substrate complexation, indicates the participation of the phosphonate functions in the binding process. This is further supported by a drastic salt effect; in 0.5 M aqueous tetrabutylammonium bromide, the binding constant between **1d** and **6** dropped to almost zero.

The complexation-induced ^{1}H NMR shifts of the substrate protons constitute a very sensitive probe of the complex structure. For example, in the dye **3**, the resonance signals arising from the pyridinium-ring protons show a large upfield shift, whereas the position of the signals arising from the amino-substituted benzene moiety protons remains almost unchanged, indicating that only the pyridinium ring is included inside the cavity of **1d**. To understand why the complex **6@1d** is more stable than that of the structurally related **5@1d**, one can assume that the amide functionality in **6** may serve as an additional binding site (Scheme 3).



Scheme 3. Two low-energy structures and relative energies ΔE_{rel} (kcal mol^{-1}) of the complex **6@1d** calculated by the force-field AMBER* (H_2O) with a Monte Carlo simulation (MacroModel 7.0).

Encouraged by the results of the work with **6**, we investigated whether **1d** is also capable of forming a complex with NAD$^+$ (**7**) as substrate. In methanol, the complex precipitated. In water, no precipitation occurred, and distinct upfield shifts were observed in the signals arising from the protons of the nicotinamide subunit as well as in the adenine nucleoside of NAD$^+$ (Scheme 2), which unambiguously indicates complex formation between **1d** and **7**. A Monte Carlo simulation (MacroModel 7.0, Amber* force field[14]) leads to an energy-minimized structure of the complex **7@1d** in water, in which the nicotinamide moiety is inside the clip cavity and the adenine subunit is bound to one naphthalene side wall of **1d** (Figure 4). This double-sandwich structure

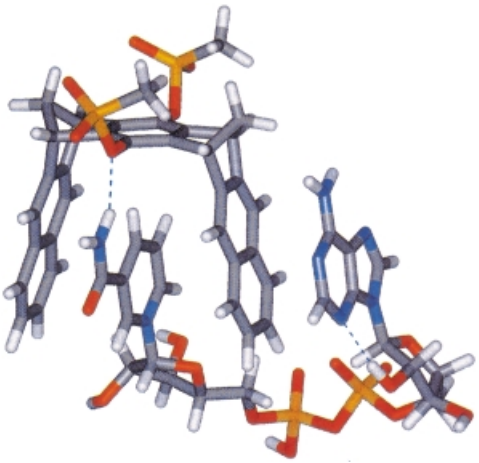


Figure 4. Structure of the complex in water between **7** and **1d** according to Monte Carlo simulations (MacroModel 7.0; AMBER*). During minimization of the energy of the complex structure, the distance between the naphthalene tips decreases from 9.7 to 7.3 Å.

explains why the proton signals of both subunits are shifted upfield in the ^{1}H NMR spectrum of the complex **7@1d**. Additional evidence for this structure comes from the relatively strong upfield shifts of the resonance signals from the protons in the sandwiched naphthalene walls of **1d** ($\Delta\delta_{max} = 0.54$ and 0.56 ppm; the average values lie between 0.2 and 0.4 ppm), which are greater than those of all other complexes. Thus, additional π-stacking and van der Waals interactions may be participating. This arrangement bears

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some similarity to the natural environment of NAD^+, which is bound in a well-defined region called the "Rossmann fold"[14] in most dehydrogenases. Adenine and nicotinamide are both buried in cavities with at least one hydrophobic side.

The K_a value for the formation of the complex **7@1d** is smaller than that for **6@1d** (Table 1); this is consistent with the fact that the anomeric region of ribose in **7** is sterically more demanding than the methyl group in **6**. In the future we plan to optimize the receptor design for highly efficient and selective NAD^+ recognition in water, for example, by eliminating one of the phosphonate moieties in **1d**, and replacing it with a neutral binding site for ribose.

We have created a molecular clip which binds *N*-alkylpyridinium ions very effectively in water. To our knowledge, this is the first time that NAD^+ has been complexed by a synthetic receptor molecule. Since not every primary *N*-alkylammonium ion is bound in methanol or water, clip **1d** is also the first artificial host that binds only to flat heteroaromatic cations such as *N*-alkylpyridinium derivatives, most other synthetic receptors for these species have a macrocyclic structure and can also accomodate quaternary ammonium salts. Although the molecular clip presented here is obviously not suited for the molecular recognition of sp^3-hybridized ammonium ions, the related molecular tweezers, which are able to bind dialkylammonium salts in organic solvents,[15] are promising candidates for this purpose.[16]

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